The impacts of urbanization on ground beetle functional traits (Coleoptera: Carabidae)

Ellen J. Dunkle

Advisors: Kayla I. Perry, and Mary M. Gardiner

April, 2019

Introduction

Urbanization is a form of environmental disturbance that destroys and fragments habitats, which has an impact on ecosystem services (Johnson et al. 2018; Liu et al. 2019). Anthropogenic disturbances not only affect the biodiversity of local biota (Johnson et al. 2018; Niemelä 2001) but can influence certain functional traits and physiological conditions of these organisms as well (Chen et al. 2018). As urban spaces continue to expand in order to accommodate growing populations (Yang et al. 2017), it is increasingly important to understand exactly how these changes are affecting flora and fauna within urban areas.

Environmental changes have been shown to shape various traits in arthropods including body size, body shape, symmetry (Beasley et al. 2017; Weller et al. 2004), and aedeagus size (Chen et al. 2018). However, not all organisms will be affected in the same way as they respond to environmental changes according to their ecological requirements, life histories, and morphological traits (Kotze et al. 2011). Arthropods provide ecologically important services including pollination, decomposition, nutrient cycling, and biological control of pests (Kotze et al. 2011; Isaacs et al. 2009), which are all linked to their functional traits (Fountan-Jones et al. 2015; Moretti et al. 2017). Many arthropods are also considered indicators of habitat quality (Kotz et al. 2011). It is thus important to fully understand the influence of urbanization on arthropods in order to guide conservation efforts and to aid the restoration of ecosystem services (Gardiner et al. 2013).

Few studies have been conducted specifically outlining the impact of urbanization on ground beetles (Carabidae), which are considered ecologically and agriculturally beneficial insects (Eskelson et al. 2011). In order to excogitate the impacts of urbanization on carabid functional traits, the objective of this study was to measure shifts in body size, aedeagus size, and fluctuating asymmetry (FA) in urban environments in comparison to rural habitats.

Literature Review

Equilibrium Theory of Island Biogeography: The equilibrium theory of island biogeography (ETIB) proposes that the number of species on any island is determined by a balance between the rate of immigration and the rate of extinction. The distance from mainlands and size of islands influence these rates, as closer islands are more easily colonized (barriers to entry are few) and larger islands have the potential to harbor more species (Wilson, 1967). This theory is easily adjusted in order to correspond with urban environments in which the rural surroundings act as mainlands and the green spaces within cities fulfill the profile of islands. Furthermore, the "sea of concrete" in built up areas may pose significant barriers to entry into urban green spaces (Fattorini et al. 2018; Kotze et al. 2011; Weller et al. 2004). This is especially evident for small animals such as insects that are capable of establishing metapopulations in diminutive environments (Jones et al. 2012).

Isolation: The isolation posed by this model of biogeography can be a driver of natural selection in the presence of reduced gene flow (Santangelo et al. 2018; Munshi-South et al. 2016; Keller et al. 2003) causing gradations in certain characteristics (Vasemägi 2006). Reduced genetic variation caused by shrinking populations has the ability to lead to allopatric speciation over time (Okuzaki et al. 2015; Wilson, 1967). In the short-term, this can provoke inbreeding, resulting in less fit offspring and/or aberrant characteristics (Gangloff 2017; Keller et al. 2003). Local adaptations as a result of urban environments have been shown to occur even in highly mobile species (high gene flow) such as the red-tailed bumble bee *Bombus lapidarius* (Linnaeus, 1973) (Panagiotis et al. 2018). Because the barriers to entry are more difficult to cross for flightless individuals including many ground beetle species, local adaptation could have a more pronounced effect on such species.

Asymmetry: Physiological conditions of carabids are quick to acclimate to disturbed environments and can be used as a measure of habitat quality (Elek et al. 2017). Fluctuating asymmetry (FA) is a particularly useful measure of environmental conditions (Elek et al. 2017; Cuevas-Reyes et al. 2013) and has been demonstrated in some carabid species to increase towards city centers, indicating strong environmental stressors (Weller et al. 2004) possibly due to pollution levels. High levels of asymmetry can also result from interbreeding following isolation via genetic drift (Idrisova et al. 2016) and can be used to support the concept that urban green spaces act as islands in accordance with ETIB (Weller et al. 2004)

Body Size: Areas of high disturbance have been shown to drive the prevalence of smaller individual carabid beetles within cities, particularly for detritivore species in which competition for food is more severe (Tyler 2010; Weller et al. 2004; Niemelä et al. 2002; Ulrich et al. 2008). Smaller individuals may also prevail in urban areas because they are more commonly macropterous, while larger species typically have reduced wings if any, thereby restricting their dispersal ability and increasing isolation (Sadler et al. 2006). Furthermore, a decrease in size may be a response to warming temperatures either caused by climate change and/or the heat island effect, with larger insects being more susceptible (Tseng et al. 2018; Oke et al. 1982, 1973). This may have serious consequences for these insects as body size impacts fitness via female fecundity and male mating success, both of which typically increase with increased body size (Okuzaki et al. 2017; Andersson 1994; Honěk 1993; Wiklund et al. 1988; Alcock et al. 1983).

Aedeagus Size: Insect genitalia are complex and more rapidly evolving than external traits (Macagno et al. 2011) exhibiting negative static allometry in which smaller male individuals tend to have larger genital size, holding the converse to be true as well (Eberhard et al. 1998). Therefore, provided a change in body size, it is possible that a shift in aedeagus size may correlate (Okuzaki et al. 2015, 2014). The ground beetle *Carabus masuzoi* (Imura et al., 1989) has displayed diminished aedeagus size within disturbed forests of Taiwan due to extensive thinning (Chen et al. 2018). Many factors may drive these differences in male genital size including sexual selection, random effects of dispersal, and pleiotropy (Chen et al. 2018). Both sexual selection and dispersal capabilities can be directly influenced by urbanization as a result of the isolation posed by habitat fragmentation (Adler et al. 2013).

Hypotheses and Predictions

By applying a functional trait-based approach to ETIB, the following hypotheses were developed in order to test the impacts that urbanization has on ground beetle traits and their abilities to cross over from rural mainlands into urban islands:

1. *Urbanization poses barriers to ground beetles resulting in shifts in body size.*
2. *Urbanization poses barriers to ground beetles resulting in shifts in fluctuating asymmetry.*
3. *Urbanization poses barriers to ground beetles resulting in shifts in aedeagus size.*

Corresponding predications follow:

1. *Smaller beetles will be more prevalent in urban environments while larger beetles will dominate rural habitats.*
2. *Fluctuating asymmetry will be higher in urban areas than in rural areas.*
3. *Corresponding with changes in body size, aedeagus size will be higher in urban areas and smaller in rural areas.*

Materials and Methods

Study Organisms: Carabid beetles are often used as bioindicators of habitat quality as their physiology is quick to respond to environmental disturbances (Beckers et al. 2017; Skalski et al. 2016; Koivula et al. 2011; Beaudry et al. 1997) as well as climatic changes (Tseng et al.

2018). Their taxonomy and ecological requirements are also well documented (Niemelä et al. 2002) and sampling is simple, making them ideal study organisms.

Study Sites: Cleveland, Ohio is considered a shrinking city as it has experienced a loss of over half of its human population since the 1950s (Blanco et al. 2009). This has led to the demolition of thousands of abandoned residential buildings resulting in the creation of vacant lots which are minimally managed green spaces (Cleveland Land Lab, 2008). This coupled with the abundance of rural surroundings and forested metro parks makes it a suitable area for comparing multiple aspects of urban and rural habitats.

Ground beetles were collected from 40 sites within the city of Cleveland, Ohio and its surrounding rural areas (Fig. 1). These sites exhibit 5 distinct habitat treatments (Fig. 2A-E)

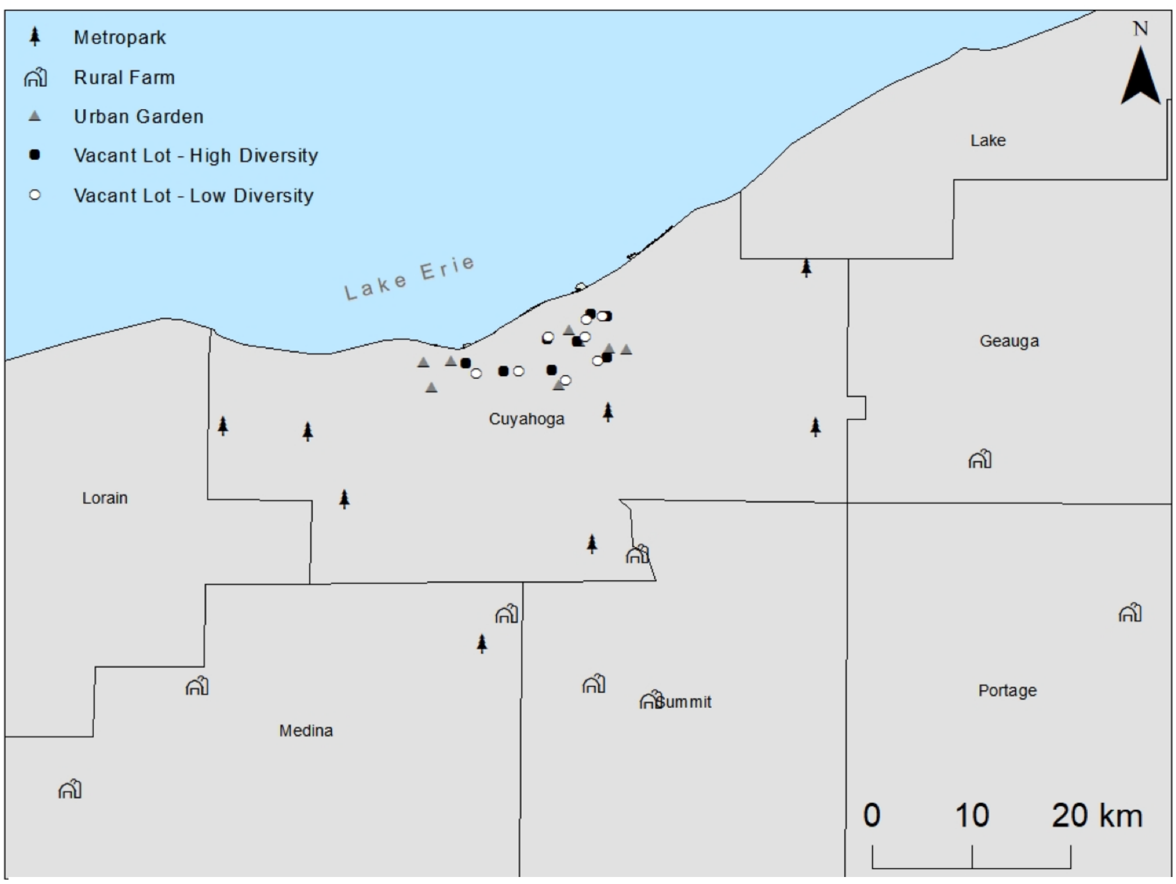

Figure 1. Map of all 40 collection sites from Cleveland, Ohio and surrounding rural areas. © Emily Sypolt 2018.

including vacant lots mown monthly, pocket prairies seeded with 2 native prairie grasses and 16 native wildflowers in November 2014, both urban and rural agroecosystems and finally, rural metro park forests.

Sampling: Four unbaited 1L pitfall traps (Fig. 2F) were employed in 8 sites per treatment with soapy water to collect ground beetles monthly between June and August of 2018. Sampling intervals were 31 May-14 June, 11-26 July, and 6-14 August. Ensuring proper collection of forest communities, pitfalls were installed between 40 and 50 meters into the forest at metro park sites with an additional two pitfalls at the forest edge. The contents of each trap were placed in alcohol and transported to a lab to be sorted from other ground-dwelling arthropods and identified to species using Bousquet, 2010 and Lindroth, 1961-1969 dichotomous keys. The results of this paper focus on the four species (Fig. 3) that were found in highest

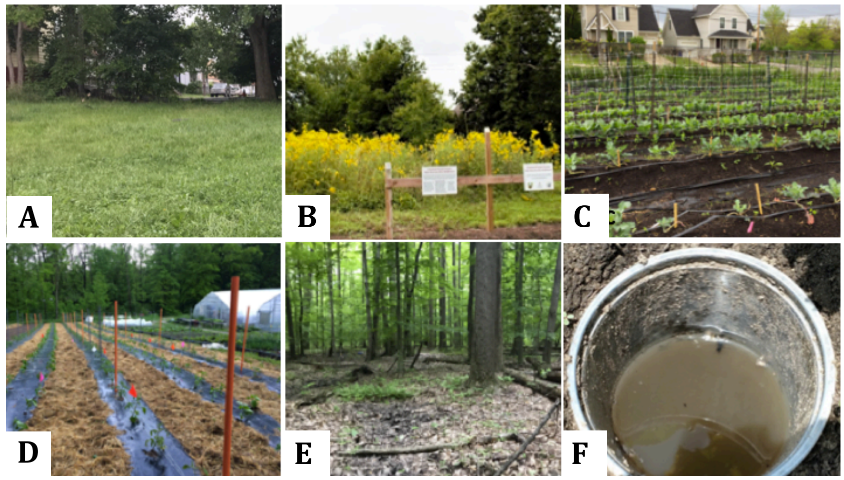

Figure 2. The five treatments where beetles were collected. **A**. Vacant lot mown monthly; **B**. Pocket prairie seeded with wildflowers; **C**. Urban garden; D. Rural farm; **E**. Rural metro park forests and **F.** 1L pitfall trap placed just beneath soil surface.

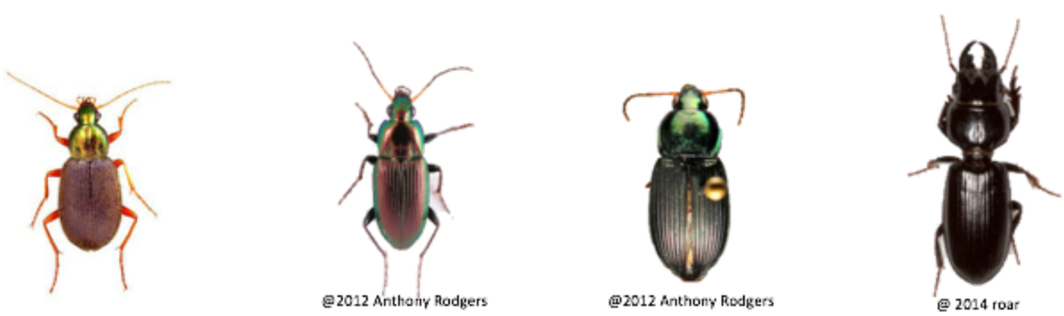

Figure 3. The four carabid species collected in most abundance including (from left to right: *Chleanius tricolor, Poecilus chalcites, Poecilus lucublandus,* and *Scarites vicinus.*

abundance including *Chlaenius tricolor* (Dejean), *Poecilus chalcites* (Say), *Poecilus lucublandus* (Say), and *Scarites vicinus* (Chaudoir).

Measurements: All measurements (mm) were taken following the preservation of pinned and dried specimens using a digital caliper. All individual traits were measured thrice and averaged for accuracy. Body size was measured for both sexes following the technique outlined by Okuzaki and colleagues (2015) from the front margin of the labrum to the apical portion of the elytra. Beetles were boiled in order to prevent damage followed by the measurement of aedeagus length from base to tip (Fig. 4). In order to test the degree of asymmetry, each elytrum was measured, and the absolute difference was calculated. In accordance with the methods outlined by Weller and colleagues (2004) the relative Fluctuating Asymmetry was calculated as the percentage of this absolute difference in relation to overall body size with the following equation:

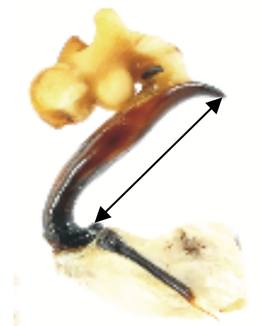

Figure 4. Beetle aedeagus with arrow indicating measurement from base to tip. Modified from Chen et al. 2018.

$$FA = \frac{(R - L)}{(2 \times (R + L))}$$

where *FA* is the Fluctuating Asymmetry, *R* is the average length of the right elytrum following three measurements and *L* is the average length of the left elytrum following three measurements.

Vegetation data was collected from each site using six 1m^2 quadrats that were randomly selected from three 10m transects. Data was recorded during the same dates in which beetles were collected from traps in June, July, and August. Within each transect, percentage cover was estimated as well as the total number of different plant species, though the species were not identified. Information from each transect was then compiled to create a composite record of percentage ground cover and species richness for each collecting location.

Heavy metal contamination in soil was used as an indicator of environmental stress on ground beetles. Soil cores were were collected during the summers of 2016-2017. Three cores (10 cm in diameter x 5 cm deep) were taken from random locations in each site and combined to form a single soil sample of the individual collecting location. The samples were then dried at 70°C for 72hrs and filtered using a 2mm sieve. Total concentrations (µg/g) of heavy metals including aluminum (Al), antinomy (Sb), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), vanadium (V), and zinc (Zn) were all quantified. These heavy metal levels were analyzed using EPA 6200 XRF method in the Soil Water and Environmental Laboratory (SWEL; https://swel.osu.edu/) at Ohio State University.

Background heavy metal concentrations determined for the eastern US (US EPA 2007) were used to calculate the ratio of observed heavy metal contamination at each site versus these background levels. An integrated pollution for each site was then determined and the contamination Factor (CF) (Hakanson 1980, Loska et al. 2004, Weissmannová et al. 2017) calculated for each metal at the specific collecting location with the following equation:

$$CF = \frac{Cs_i}{Cb_i}$$

where Cs_i is the measured concentration of heavy metal i in the soil sample, and Cb_i is the regional background concentration of the corresponding metal. Background concentrations (µg/g) for As were 5.0, 71000.0 for Al, 1.0 for Sb, 350.0 for Ba, 0.23 for Cd, 45.0 for Cr, 18.0 for Cu, 21000.0 for Fe, 19.0 for Pb, 430.0 for Mn, 15.0 for Ni, 60.0 for V, and 45.0 for Zn (US

EPA 2007). *CF* classes outlined by Hakanson (1980) were used to evaluate contamination levels of individual heavy metals and are classified as follows: low contamination (CF < 1); moderate contamination (1 ≤ CF < 3); considerable contamination (3 ≤ CF < 6); and very high contamination (6 ≤ CF). Finally, for each site the Pollution Load Index (PLI) (Tomlinson et al. 1980, Liu et al. 2005, Weissmannová et al. 2017) was calculated with the following equation:

$$PLI = \sqrt[n]{CF_1 \times CF_2 \times CF_3 \ldots \times CF_n}$$

where *n* is the number of metals investigated, and *CF* is the contamination factor for each metal at the site. Four PLI classes have been used to evaluate site-level contamination of heavy metals and are classified as follows: low contamination (PLI < 1); moderate contamination (1 ≤ PLI < 2); considerable contamination (2 ≤ PLI < 3); and very high contamination (3 ≤ PLI) (Wang et al. 2010, Demková et al. 2017).

Statistics

All statistical analyses were completed using the 'stats' package in R version 3.5.1 (R Core Team 2019). Ground beetle response variables were tested for assumptions of normality and homogeneity of variance. Non-parametric tests were used when variables did not meet these assumptions.

Interspecies analysis: Community-weighted mean (CWM) for body size was calculated for the four ground beetle species using the dbFD function in the package ‘FD’ (Laliberte et al. 2014) in R version 3.5.1 (R Core Team 2019). A Kruskal-Wallis non-parametric test was then used to compare the CWM between treatment types and a Spearman correlation analysis to test the CWM body size across the urban-rural gradient. This gradient was determined by the distance of each site from the city center as determined using ArcGis (41.499301°N;

-81.694402°W). A Kruskal-Wallis test was again implemented in order to test the FA of the four species in accordance with each treatment. Another Spearman correlation analysis was used to test the FA of the four species along the urban-rural gradient.

Intraspecies analysis: The only species found in all treatments was *Chlaenius tricolor,* while the other three abundant species were restricted from urban spaces. Therefore, *C. tricolor* is the focus of evaluating the intraspecies variation in the aforementioned functional traits as they relate to the five treatments. Much like the interspecies evaluation, Kruskal-Wallis non-parametric tests were used to compare the aedeagus size and FA of *C. tricolor* to each treatment type. For body size analysis of *C. tricolor*, the data were tested to be normal and an Analysis of Variance (ANOVA) was used to evaluate these measurements in comparison to each treatment.

Heavy metals and vegetation: Kruskal-Wallis non-parametric tests were used to compare percentage cover of vegetation, plant richness, and the Pollution Load Index among treatments. Spearman correlation analyses were used to assess the relationship between ground beetle traits (CWM for body size and FA for each species) and environmental variables (PLI, percentage cover of vegetation, and plant richness).

Results

The average body size of each of the four most abundant species was highly variable as was their prevalence amongst treatment types (Table 1). *C. tricolor*, of moderate size, was the only species that was found amongst all treatments, though it was caught most within the pocket prairies and far fewer were found outside the city. This is in contrast to the other three species that were confined to rural sites, including the largest species by far, *S. vicinus* and the other two moderately sized species, *P. lucublandus* and *P. chalcites*. No one species was caught in great abundance within the urban gardens.

Table 1. Number of individuals of each species caught in each treatment as well as the range of body size and average body size for each species.

Species	Vacant Lot	Pocket Prairie	Metro Park Forest	Rural Farm	Urban Garden	Body size range (mm)	Avg Body size (mm)
Scarites vicinus	0	0	3	42	0	19.5-24.0	21.7
Poecilus lucublandus	0	0	38	11	0	11.0-13.7	12.6
Poecilus chalcites	0	0	0	50	0	9.7-12.8	11.4
Chlaenius tricolor	31	57	3	11	2	10.8-13.6	12.3

Body size: The CWM for the body size of all four species revealed larger beetle body size in metro park and rural farms in comparison to urban treatments (X^2=13.9, P=0.007) (Fig. 5A). This was due to the prevalence of the largest species, *S. vicinus* in these rural areas.

Body size of *C. tricolor* was similar among treatments (F=1.64, P=0.169) (Fig. 5B), though females were larger than males. This lack of significance was likely due to the high variability of individual size within the city.

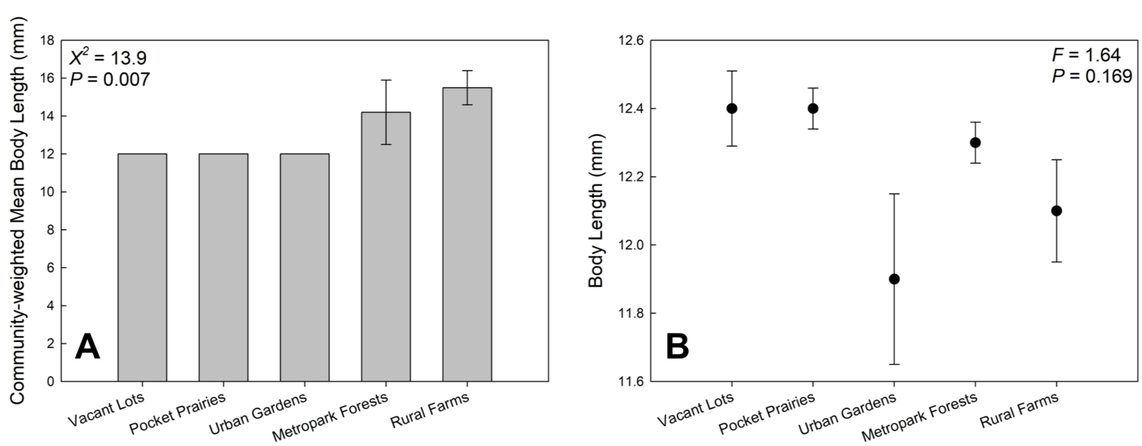

Figure 5. A. CWM body size (mm) of all four species combined in each treatment. No error bars are present for vacant lots, pocket prairies, and urban gardens because individual variance is not shown as only *C. tricolor* was found in these sites. **B**. Average body size (mm) of *C. tricolor in* each treatment.

CWM for body size was positively correlated with increasing distance from the city center along the urban-rural gradient (rho=0.76, P<0.001) (Fig. 6A) and negatively correlated to increasing Pollution Load Index (rho=0.20, P=0.002) (Fig. 6B).

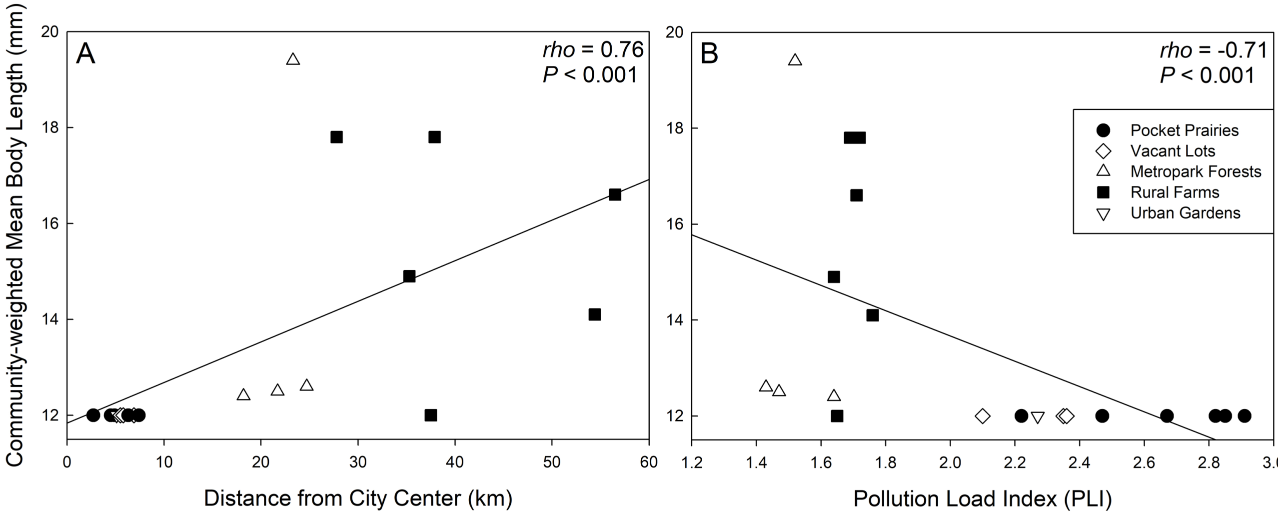

Figure 6. A. CWM body length (mm) of all four species combined at each site compared to distance from city center (km). **B.** CWM body length (mm) of all four species at each site compared to PLI.

Fluctuating Asymmetry: When evaluating FA of all species in relation to treatments, pocket prairies (b) had a significantly higher level of FA while there was no statistically significant difference in FA of other treatments (a) (X^2=18.4, P=0.001) (Fig. 7A). Levels of asymmetry within *C. tricolor* individuals was found to be higher within pocket prairies and metro park forests than other treatments (X^2=10.6, P=0.032) (Fig. 7B). While these values indicate a significant relationship, the high variability of FA resulting in large error bars makes this pattern unclear.

Furthermore, FA of all species is shown to decrease with increased distance from city center (*rho*=-0.21, P=0.001) (Fig. 8A). However, this weak relationship gradually occurs across the urban-rural gradient. Finally, in accordance with a higher PLI within the city, FA of all species increases with increased PLI (*rho*=0.20, P=0.002). (Fig. 8B), although this relationship was weak.

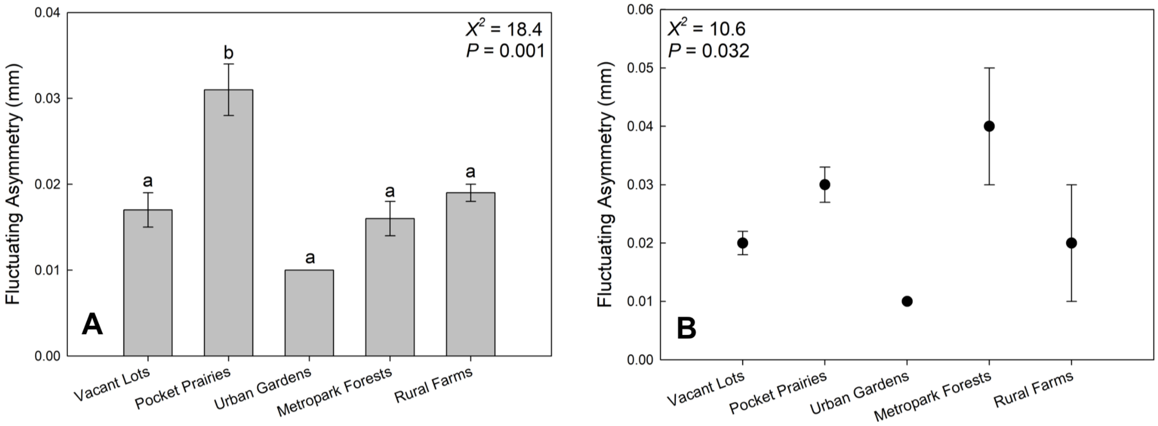

Figure 7. A. FA of all four species compared to treatment. Bars labeled a had no significant difference while the bar labeled b had significantly higher FA. **B.** FA of *C. tricolor* compared to treatment. High error bars indicate high variability due to a small sample size.

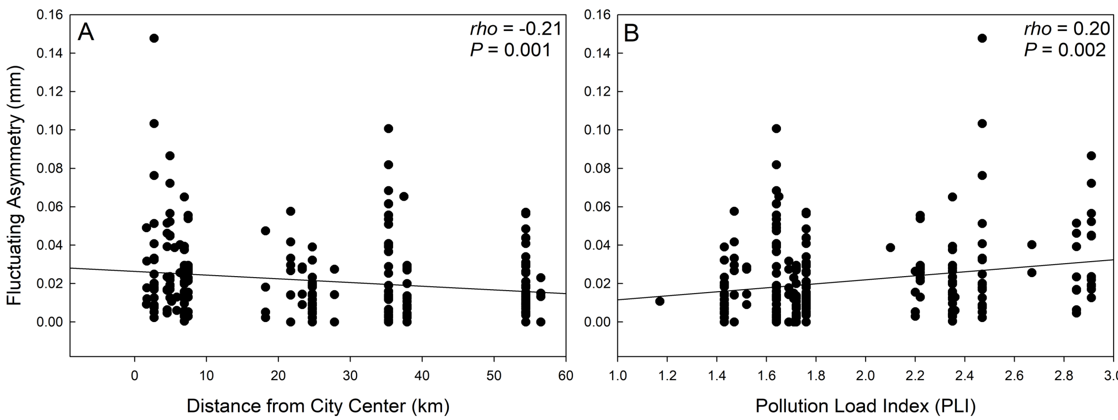

Figure 8. A. FA (mm) of all four species compared to distance from city center. **B.** FA (mm) of all four species compared to PLI.

Aedeagus Size: Comparison of aedeagus size between treatments was only applicable to *C. tricolor* as this was the sole species found in all treatments. Reproductive structures are species specific, making an interspecies analysis of aedeagus size delusive. Results revealed a trend for reduced aedeagus size of *C. tricolor* in vacant lots and pocket prairies than in other treatments (X^2=9.05, P=0.059) (Fig. 9).

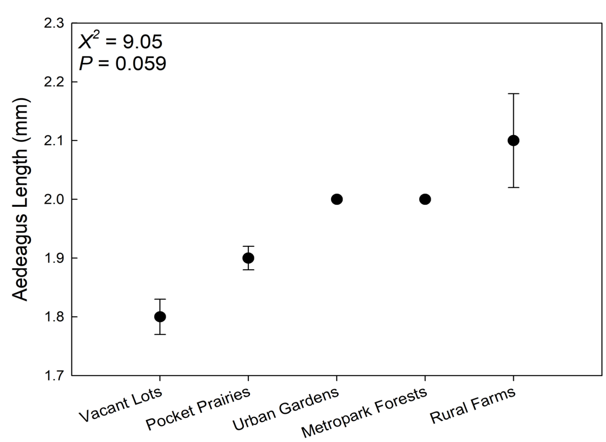

Figure 9. Aedeagus length (mm) of *C. tricolor* compared to treatment. Only one aedeagus was measured where no error bars are present.

Vegetation: Estimated plant richness based on species per site was significantly higher within pocket prairies (a) than other treatments (Fig. 10A). There was no significant difference between species richness in pocket prairies and vacant lots (ab). The remaining three treatments showed little variation in species richness (b) (X^2=13.9, P=0.007). Estimated percentage ground-level vegetation coverage (Fig. 10B) revealed a higher percentage in both vacant lots and pocket prairies (a) compared to the remaining three treatments. Furthermore, rural farms (c) had less percentage ground cover than pocket prairies and vacant lots but they did have a statistically significant higher ground cover than urban gardens and metro park forests (b) (X^2=31.6, P<0.001).

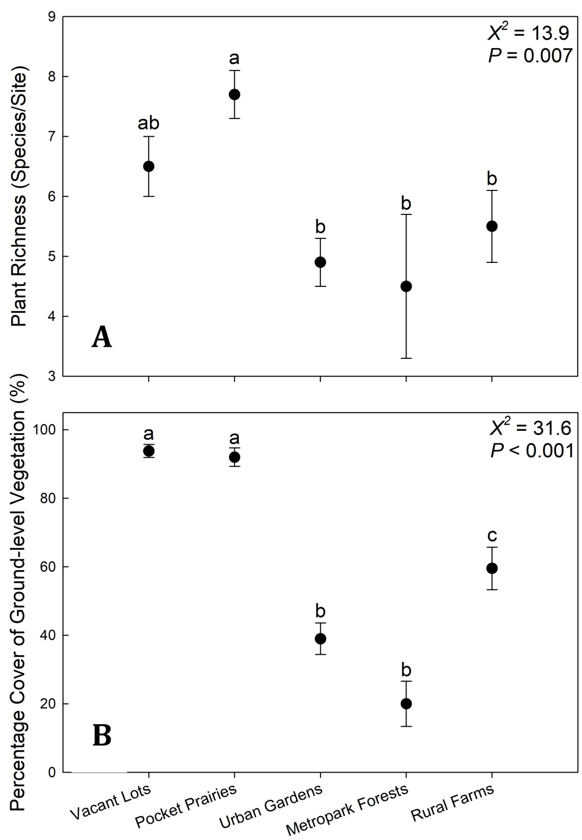

Figure 10. A. Plant richness based on number of species per site compared to treatment. **B.** Percent vegetative ground cover compared to treatment.

Heavy metals and vegetation: The PLI analysis revealed significantly higher heavy metal contamination within pocket prairies and vacant lots than other treatments (X^2=212.7, P<0.001) (Fig. 11).

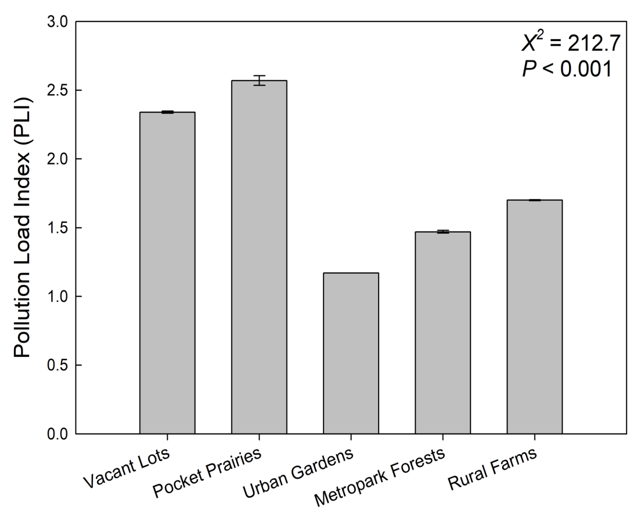

Figure 11. Pollution Load Index (PLI) for each treatment based on heavy metal contamination of soil samples.

Discussion and Conclusions

Various morphological and physiological traits of arthropods have been shown to shift in the face of environmental disturbances such as those caused by urbanization (Beasley et al. 2017; Weller et al. 2004). We adjusted the Equilibrium Theory of Island Biogeography to describe urban green spaces as islands and their rural surroundings as mainlands (Fattorini et al. 2018). We then applied a functional trait-based approach to the theory in order to gain a better understanding and to make predictions about how body size, symmetry, and aedeagus length of carabid beetles may change in the face of urbanization. Our results demonstrate that urbanization drives the prevalence of smaller carabid species in urban islands as well as an increase in fluctuating asymmetry, which are both correlated with heavy metal contamination of soil within cities. Small sample size and uneven distribution of species amongst treatments resulted in unclear associations between aedeagus length and urbanization.

As was demonstrated in *Carabus nemoralis* (O. F. Müller, 1764) by Weller et al. (2004), there was a strong relationship between increased body size and distance from city center of the four beetle species combined. This paired with an elevated PLI within cities suggest that the heavy metal contamination may be a significant stressor to carabids. These results support the theory of ETIB as it applies to urban spaces in that cities may pose barriers to larger species crossing over from rural mainlands. Therefore, our first hypothesis that urbanization causes shifts in body size is supported. However, the hypothesis cannot be supported nor refuted on an intraspecies level. The lack of significance between *C. tricolor* body size and treatments is likely due to a high variability of size caused by the presence of larger females. This is possibly exacerbated by the prevalence of more individuals within the city than in rural treatments, as they thrive in areas of human activity (Larochelle et al. 2003). A characteristic behavior of this species is the laying of eggs in mud cells that they stick to the stems of grasses (Larochelle et al. 2003), which may explain the higher number of *C. tricolor* individuals in pocket prairies, where there is more foliage and taller grasses than other city sites.

While the relationship between treatments and FA is unclear on the intraspecies level when evaluating *C. tricolor*, a marginal relationship is seen when comparing all four species. FA of the four species decreased with increased distance from city center and increased with increased PLI. This is in accordance with the findings of Weller et al. (2004), where the FA of some carabid species revealed a negative correlation with distance to city center. While our findings present a gradual trend, the statistical significance supports our second hypothesis that urbanization causes shifts in FA and the corresponding prediction that FA will increase with urbanization. The positive correlation between FA and PLI, further suggests heavy metal contamination as an environmental stressor.

While *C. tricolor* was found in all habitats, the species prevalence was not evenly distributed throughout treatments. This, as with body size and FA, led to indeterminate intraspecies analysis of aedeagus size as it relates to urbanization. Due to this inconsistency, our third hypothesis that aedeagus size would be affected by urbanization can neither be supported nor refuted. This is in contrast to the findings of Chen et al. (2018) where the aedeagus of certain carabid species had been demonstrated to be smaller in disturbed environments. However, Chen and colleagues were observing this trend in thinned forests rather than urban green spaces. A higher sample size and/or the evaluation of a species with a more even distribution of individuals would likely lead to more definitive results. An additional season of collection could supply more information in order to better test this hypothesis.

This study supports the concept that ETIB can be used to form hypotheses and predictions about geographical trends in urban green spaces as was suggested by Fattorini et al. (2018). In order to further test how well ETIB describes urban trends and its impacts on the functional traits of local biota, further research is necessary. Investigation into landscape characteristics should be evaluated such as the connectivity between locations, the amount of impervious space that may be difficult for small arthropods to cross, and the size of sites in both urban and rural areas could help us to better understand the barriers faced by arthropods in urban environments. Furthermore, this study does not elucidate the underlying causes resulting in disparate body size and FA. Though heavy metal contamination appears to be a contributing factor, further evaluations of treatment characteristics such as air pollution, temperatures, and amount of available resources could help demonstrate the driving force of these trends. Finally, our assessments do not consider habitat isolation that could impact the genetic makeup of individual beetles. Future studies evaluating potential genetic changes along these urban-rural gradients could address the extent of genetic isolation that urban ETIB poses to ground beetles and other arthropods.

Acknowledgments

A special thanks to the Cleveland Metro Parks, Cleveland Botanical Gardens, and all Cleveland residence and rural farmers for allowing us to collect in your spaces. Funding provided by USDA NIFA Food, Agriculture, Natural Resources and Human Sciences Education and Literacy Initiative Postdoctoral Fellowship (2018-67012-28011) to K.I.P., and a Division of Environmental Biology CAREER Grant (CAREER-1253197) and USDA Agricultural Research Program Initiative Foundational Programs Grant (2017-67013-26595) to M.M.G.

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